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Phytochemical Screening and Antihyperglycemic Activity of *Basella rubra*

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Abstract

The objective of the present study was phytochemical screening and antihyperglycemic activity of Aqueous extract of *Basella rubra* in streptozotocin-induced diabetic rats. The phytochemical screening of *Basella rubra* was done by using different methods. *Basella rubra* is found to contain high levels of the non-enzymic ,enzymic antioxidants, and Phytonutrients it would be effective in treating the diseases caused by free radicals and also in the treatment of diabetes.

The STZ induced diabetic rats are divided into four groups of six animals each. Group I served as Non diabetic control, Group II - Diabetic control, Group III Non-diabetic rats treated with *Basella rubra,* Group IV Diabetic rats treated with *Basella rubra*. The Aqueous extract of *Basella rubra* produced a significant antihyperglycemic (decrease in blood glucose level) effect ($p<0.05$) after the treatment, and decreased level of fructosamine .The findings of the present study suggest that it is rich in phytonutrients, it includes the enzymic and nonenzymic antioxidant. *Basella rubra* being a good source of these enzymes may be effective in the treatment of hyperglycemia .From this study it was concluded that aqueous extract of *Basella rubra* produced significant antihyperglycemic activity in STZ induced diabetic rat which is comparable to control rat.

Key Words: Phytonutrients, *Basella rubra* , Diabetes, Antioxidant, Hyperglycemia

Introduction

Diabetes Mellitus (DM) is a chronic disorder resulting from a number of factors in which an absolute or relative deficiency of insulin or its function occurs. It is projected that by the year 2025, India alone would have 57 million diabetics mainly of type 2 diabetes constituting 90% of the diabetic population [1]. DM is grossly reflected by profound changes in protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs [2]. Increased urea nitrogen production in DM may be accounted for by enhanced catabolism of both liver and plasma proteins. Management of DM without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs (eg. sulphonylureas and biguanides) have undesirable side effects [3]. Medicinal plants are a good source of natural antioxidants believed to exert their effect by reducing the formation of the final active metabolite of the drug-induced systems or by scavenging the reactive molecular species to prevent their reaching a target site [4].

Basella rubra known as Malabar spinach is also known as cyclone spinach. It belongs to *Basellaceae* family. It is a climbing perennial plant. Malabar spinach has thick tender stems and the leaves are almost circular to ovate, alternate and short petioled. They are thick, rugose, succulent and colored from green to purple. The leaves are used in catarrhal affection and to hasten suppuration. Decoction of the root relieves bilious vomiting [5]. In general, spinach leaves contain several active components including flavonoids exhibit antioxidative, antiproliferative and anti-inflammatory properties in biological system. Spinach extracts have been demonstrated to exert numerous beneficial effects such as chemo and central nervous system protection, anticancer and antiaging function [6]. However, available literature shows that no experimental work has been carried out to verify the claims on the anti-diabetic effect of *B. rubra*. Therefore, it was considered worth while to undertake this study to evaluate the phytochemical screening and anti-diabetic activity of *B. rubra* in streptozotocin-induced diabetic rats.

Materials and Methods

Plant materials

B. rubra plant was collected from the village area (Dindigul district,Tamil Nadu, India) and raised in the university campus under normal climatic conditions (35 - 37°C). The plant was identified and authenticated (No.BSI/SC/5/21/04-05/Tech.367) from Botanical Survey of India (BSI), Tamil Nadu Agriculture University (TNAU) Coimbatore. Fresh leaves were collected for the study whenever required.

Preparation of the plant sample

Fresh leaves of *B. rubra* (400 mg) ground with 10 ml water in a mortar and pestle. The grounded material was utilized for the experiment.

Animals and treatment

Male albino rats were selected for the study and experiments were conducted as per Ethical guidelines. They were of the same age and weight (150 – 200 gm). The rats were housed in polycarbonated clean cages under a 12 /12 h normal light/dark cycle. The animals were fed with standard diet and water *ad libitum*. After keeping in the laboratory condition for a week for acclimatization the experiment was initiated. A total of 24 rats were categorized into 4 groups, each group consisting of six rats.

Group I: Was normal healthy control.

Group II: Diabetic control, diabetes was induced in rats after 18 h fasting by intraperitoneal administration of streptozotocin (60 mg/kg body weight dissolved in 10 mM citrate buffer pH 4.5) [7]. STZ injected animals exhibited massive glycosuria and hyperglycemia within few days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration. A blood glucose level of 200 mg/dl was considered a diabetic.

Group III: Rats are non diabetic rats treated with *B. rubra*.

Group IV: Diabetic rats administered with *B. rubra*.

The *B. rubra* paste *(*400 mg/100 gm body weight) was orally gavaged to group III and IV daily for 30 days. After 30 days of treatment, the blood samples were collected after overnight fasting and it was used for separating the serum for analyzing the biochemical parameters.

Phytochemical Screening

Phytochemical screening of *Basella rubra* was done by using different methods. Moisture content, crude fiber, Potassium level was done by (Raghuramulu *et al*.) [8], Chromium (Krishna and Ranjan) [9], Catalase (Luck) [10], Peroxidase (Reddy *et al*.) [11], Superoxide dismutase (Misra and Fridovich) [12], Ascorbic acid(Roe and Kuether) [13], Vitamin E (Rosenberg) [14], Carotenoids (Zakaria) [15], Glutathione (Moron *et al.*) [16].

Biochemical parameters

Glucose is an indispensable energy supplying substrate, which supports cellular function and is an important marker in diabetes. So in the present study blood glucose was estimated in control as well as in diabetic animals by the method of Raghuramulu et al.*)*[17] and Fructosamine (Armbruster) [18].

Result and Discussion

Quantitative detection of phytochemicals in the leaves of Basella rubra

The amount of nutrients present in *Basella rubra* was analysed and it is given in Table I.

Table I: Phytonutrients in *Basella rubra*

S.No.	Nutrient	Quantity
1	Moisture content (g/100g)	81.0 ± 1.63
2	Fibre content (g/100g)	25.3 ± 2.10
3	Potassium (mg/100g)	204.8 ± 1.77
4	Chromium (mg/100g)	0.004 ± 0.001

Values are mean ± SD of six replicates

Basella rubra contains appreciable quantity of moisture, fiber content, and minerals such as potassium and chromium. The fibre content of *B. rubra* is 25.3 g/100g. Fibre is found to be good for diabetic patients. The water soluble fibre can help improve blood sugar control and lower the level of cholesterol.

Supplementation of potassium improves insulin sensitivity, responsiveness and secretion; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart diseases and atherosclerosis [18].

B. rubra is also found to contain chromium (0.004mg/100g). Chromium is vital to proper blood sugar control as it functions as a key constituent of the "glucose tolerance factors". In diabetes, supplementing the diet with chromium has been shown to decrease fasting glucose levels, improve glucose tolerance, lower insulin levels and decrease total cholesterol and triglyceride level while increasing HDL cholesterol [19]. Chowdhury and Bhatacharya [20] have also reported that the micronutrient chromium is known to play a role in carbohydrate metabolism, more specifically in insulin action. Supplementation with trivalent chromium often causes a modest improvement of glycaemic control in diabetic patients.

Enzymic antioxidants

The activities of various enzymic antioxidants in *Basella rubra* are presented in Table II.

Table II: Enzymic antioxidants in *Basella rubra*

S.No	Enzymic antioxidants	Activity (U/mg protein)
1	Catalase *	6.30 ± 0.200
2	Peroxidase **	0.40 ± 0.025
3	Superoxide dismutase ***	6.90 ± 0.258

Values are mean ± SD of six replicates

* Amount of enzyme that brings about a decrease in absorbance of 0.05 at 240 nm

** Change in absorbance/min/mg protein

*** Amount of SOD that causes 50% reduction in the extent of NBT oxidation

From the above table it is clear that the enzymic antioxidants catalase, peroxidase, superoxide dismutase are high in *Basella rubra*.

Arivazhagan *et al*. [21] have reported that SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical ($O_2^{\bullet-}$), which damages the membrane and biological structure.

Catalase has been shown to be responsible for the detoxification of significant amounts of H_2O_2 .SOD and catalase are two major scavenging enzymes that remove the toxic free radicals *in vivo*. Reduced activity of SOD and catalase in liver and kidney has been observed during the diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide [22].

Basella rubra being a good source of these enzymes may be effective in the treatment of hyperglycemia.

Non-enzymic antioxidants

The non-enzymic antioxidants in *Basella rubra* were assessed and illustrated in Table III.

Table III: Non-enzymic antioxidants in *Basella rubra*

S.No	Non-enzymic antioxidants	Quantity (mg/g)
1	Ascorbic acid	31.0 ± 1.290
2	Vitamin E	4.51 ± 0.260
3	Carotenoids	5.6 ± 0.157
4	Glutathione	10.03 ± 0.022

Vaues are mean ± SD of six replicates

From the above table, it is evident that *Basella rubra* is a good source of non-enzymic antioxidants such as ascorbic acid, and glutathione, whereas vitamin E and carotenoids are present in low amounts.

It has been confirmed that vitamin C protects the body from damage due to inflammation, and high doses of vitamin C can improve the immune function [23].

Vitamin E is an important antioxidant in the cell membrane [24]. Metin et al. [25] have reported that vitamin E (α-tocopherol) is one of the most important and least toxic of all lipid soluble antioxidant vitamins. It scavenges peroxyl radical intermediates in lipid peroxidation and is responsible for protecting polyunsaturated fatty acids (PUFA) present in cell membrane and low-density lipoprotein against lipid peroxidation.

Effect of Basella rubra on blood glucose

Glucose levels were found to be significantly increased after STZ administration, and there after decreased by administration of *Basella rubra (*Nirmala and saroja [26]*.* Decrease in serum glucose may be due to the regeneration of beta cells of the pancreas, which were destroyed by STZ. Administration of *Basella rubra* extract produced a significant ($p<0.01$) decrease in the blood glucose as compared to diabetic control (Table -IV) Our results are corroborative with the report of Lemhadri et al., [27], who showed that administration of the aqueous extract of *Origanum vulgare* to STZ-induced diabetic rats caused a significant reduction in the blood glucose level.

Table –IV: Blood glucose level in experimental rats.

Group	Treatment	Glucose (mg/dl)
I	Non-diabetic control	80.0 ± 3.65^{a}
II	Diabetic control	252.0 ± 4.16^{c}
III	Non-diabetic + *Basella rubra*	82.0 ± 4.76^{a}
IV	Diabetic + *Basella rubra*	110.1 ± 3.93^{b}
CD $_{(0.05)}$		5.46

Values are mean ± SD
Values with different superscripts differ significantly (P<0.05)

Table V: Fructosamine Levels in Experimental Rats.

Group	Treatment	Fructosamine (mg/100 ml)
I	Non-diabetic control	250.5 ± 3.94^{a}
II	Diabetic control	324.4 ± 4.02^{c}
III	Non-diabetic + *Basella rubra*	254.8 ± 8.93^{a}
IV	Diabetic + *Basella rubra*	299.7 ± 5.84^{b}
CD $_{(0.05)}$		7.9

Values are mean ± SD
Values with different superscripts differ significantly (P<0.05)

It is evident from the above table that the fructosamine level is significantly higher in the diabetic rats when compared to that of the other groups of rats. In diabetic rats treated with *Basella rubra,* the fructosamine level got reduced significantly ($P< 0.05$). However, it did not reach the control level.

This indicated that although *Basella rubra* is effective in lowering the fructosamine level, either the dose (400mg) administered or the duration of treatment is not sufficient.

Our results are consistent with that of Ryan *et al*. (2002) [28] who have also reported that fructosamine level is lowered in Type I diabetic rats that were given CMT-8 daily for 3 weeks.

From the above study it was concluded that, *Basella rubra* is found to contain high levels of the non-enzymic ,enzymic antioxidants, and Phytonutrients it would be effective in treating the diseases caused by free radicals and also in the treatment of diabetes. The results of our study suggest the leaf pulp of *B. rubra* has beneficial effects on blood glucose levels, Fructosamine. Further, pharmacological and biochemical investigations are in process to elucidate the exact chemical compound(s) responsible for the antidiabetic activity of *Basella rubra.*

References

[1] Ramachandran A, Snehalatha C, Viswanathan V., 2002. Burden of type 2 Diabetes and itscomplications - The Indian Scenario. Curr. Sci. 83: 1471-1476.

[2] Almdal TP, Vilstrup H., 1987. Effect of streptozotocin-induced diabetes and diet on nitrogen loss from organs and the capacity of urea synthesis in rats. Diabetologia.,30: 952-6.

[3] Kameswara Rao B, Appa Rao CH., 2001. Hypoglycemic and antihyperglycemic activity of alternifolium Walp. Seed extracts in normal and diabetic rats. Phytomed., 8:88-93.

[4] Kaleem M, Kirmani D, Asif M, Ahmad QU, Bano B.,2006. Biochemical effects of Nigella sativa L seeds on diabetic rats. Indian J Exp Biol.,44:745-8.

[5] Nadkarni KM.,1976. *Indian Materia Media. With Ayurvedic, Unanitibbi, Siddha ,Allopathic, Homeopathic, Naturopathic & Home Remedies, appendices and indexes,*, volume 1&2. Bombay. Popular Prakashan PVT, LTD.

[6] Lomnitski L, Bergman M, Nyska A, Ben-shaul V, Grossman S., 2003. Composition, efficacy and safety of spinach extracts, Nutr cancer.46: 222-31.

[7] Archana S, Rashmi N, Khemani LD., 2001.Hypoglycemic effect of *Hibiscus rosasinensis L*. leaf extract in glucose and streptozotocin-induced hypoglycemic rats. Indian Journal of Experimental Biology., 39: 284-286.

[8] Raghuramulu N, Nair MK, Kalyanasundaram S., 1983. A manual of laboratory techniques, I edition, National Institute of Nutrition, KMR, Hyderabad, pp31-32.

[9] Krishna G, Ranjan SK., 1991.Special Analytical Techniques in Nutritional Biochemistry, Kalyani publishers, New Delhi, 1: 61-81.

[10] Luck H.,1974. In: Methods in enzymatic analysis-2 (Ed. Bergmeyer), Academic Press, New York, p885.

[11] Reddy KP, Subhani KH, Chan PA, Kumar KB.,1995. Effect of light and benzyl adenine on dark treated growing rice leaves, II changes in peroxidase activity, Plant Cell Physiol., 26: 987-994.

[12] Misra HP, Fridovich A.,1972. Assay of superoxide dismutase, J. Biol. Chem., 247:3170-3171.

[13] Roe JH, Kuether CA.,1953. The determination of ascorbic acid in whole blood and urine through 2,4 dinitro phenylhydrazine derivative of dehydro ascorbic acid. J. Biol. Chem., 147:399-407.

[14] Rosenberg HR.,1992. Chemistry and physiology of the vitamins, Inter science publishers Inc, New York, pp 452-453.

[15] Zakaria H, Simpson K, Brown PR, Krotulovic A. Use of reversed phase HPLC analysis for the determination of provitamin A carotenes in tomatoes, J.chromatography, 1979;176: 109-117.

[16] Moron MS, De Pierre JN, Mannervik V.,1979. Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat lung and liver, Biochem. Biophys. Acta., 582:67-68.

[17] Armbruster DA.,1987. Fructosamine: Structure, analysis and clinical usefulness, Clin. Chem., 33: 2153-2163.

[18] Norbiato G, Bevilacque M, Merino R.,1984. Effects of potassium supplementation on insulin binding and insulin action in human obesity: Protein modified fast and refeeding, European J. Clin. Invest.,44: 414-9.

[19] Mooradian AD. 1994. Selected vitamins and minerals in diabetes, Diabetes Care, 17: 464-79.

[20] Chowdhury S , Bhattacharya B., 2003. Role of chromium in human metabolism, with special reference to type 2 diabetes, Science and Culture, 69: 135-138.

[21] Arivazhagan P, Thilagavathy T, Panneerselvam C., 2000. Antioxidant lipoate and tissue antioxidants in aged rats, J. Nutr. Biochem.11: 122-7.

[22] Shanthakumari P, Prakasam A, Pugalendi KV., 2003. Modulation of oxidative stress parameters by treatment with *Piper betle* leaf in streptozotocin induced diabetic rats, Indian Journal of Pharmacology, 35: 373-378.

[23] Cartmell MT , Kingsworth AN., 2000. Acute pancreatitis, Hosp. Med., 61: 383-385.

[24] Garg MC, Ojha S, Bansal DD., 1996. Antioxidant status of streptozotocin diabetic rats, Indian J Exp Biol., 34 ; 264 – 266.

[25] Metin G, Atukeren P, Gumustas KM, Belce A, Kayserilioglu A., 2002. The effect of Vitamin E treatment on oxidative strees generated in trained rats, J. Exp. Med., 198. 47-53.

[26] Nirmala A, Saroja S., 2006. Antidiabetic effect of Basella rubra in streptozotocin induced diabetetic rats. Journal of Extention and Research., 8:38-40.

[27] Lemhadri A, Zeggwagh AN, Maghrani M, Jouad H, Eddouks M., 2004. Anti-hyperglycemic activity of the aqueous extract of *Origanum vulgare* growing wild in Tafilalet region. Journal of Ethnopharmacology., 92: 251-256.

[28] Ryan EM, Lee HM, Liu Y, Comanducci R, Ramamurthy N, Golub L., 2002. Non-antimicrobial tetracycline reduces systemic biochemical markers associated with diabetic complication, Periodontal Research –therapy programe., pp6-9.